

CLAIMS

1. A secreted mature polypeptide which after maturation has protease activity, which polypeptide when expressed and before maturation comprises a heterologous pro-region, and which polypeptide:

5 (a) comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of the mature part of the polypeptide shown in SEQ ID NO: 28; SEQ ID NO: 33; SEQ ID NO: 37; SEQ ID NO: 41; SEQ ID NO: 43; or SEQ ID NO: 45;

10 (b) comprises an amino acid sequence which is at least 70% identical to the amino acid sequence of the mature part of the polypeptide encoded by the polynucleotide in SEQ ID NO: 1; SEQ ID NO: 2; SEQ ID NO: 25; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 36; SEQ ID NO: 40; or SEQ ID NO: 44;

15 (c) comprises a mature part which is a variant of the mature part of the polypeptide having the amino acid sequence of SEQ ID NO: 28; SEQ ID NO: 33; SEQ ID NO: 37; SEQ ID NO: 41; SEQ ID NO: 43; or SEQ ID NO: 45, the segment comprising a substitution, deletion, extension, and/or insertion of one or more amino acids;

(d) is an allelic variant of (a), (b), or (c);

(e) is a fragment of (a), (b), (c), or (d).

2. The polypeptide according to claim 1, wherein the heterologous pro-region is derived from a protease, preferably the pro-region is derived from an S2A or S1E protease, and most preferably it is at least 70% identical to the pro-region shown in SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 33, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53.

25 3. The polypeptide according to claim 1 or 2, which is a wildtype polypeptide, an artificial variant of a wildtype polypeptide said variant having one or more amino-acid(s) added to the C-terminus as compared to the wildtype, a shuffled polypeptide, or a protein-engineered polypeptide.

30 4. The polypeptide according to any of claims 1 - 3, which comprises at least three non-polar or uncharged polar amino acids within the last four amino acids of the C-terminus of the polypeptide

5. The polypeptide according to claim 3, wherein the one or more added amino acid(s) is (are) non-polar or uncharged.
6. The polypeptide according to claim 5, wherein the one or more added amino acid(s) is one or 5 more of Q, S, V, A, or P.
7. The polypeptide according to claim 3, wherein the one or more added amino acids are selected from the group consisting of: QSHVQSAP, QSAP, QP, TL, TT, QL, TP, LP, TI, IQ, QP, PI, LT, TQ, IT, QQ, and PQ.
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8. The polypeptide according to any of claims 1 – 7 which when expressed and before maturation comprises a heterologous secretion signal-peptide which is cleaved from the polypeptide when the polypeptide is secreted, preferably the heterologous secretion signal peptide is derived from a heterologous protease.
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9. The polypeptide according to claim 8, wherein the heterologous secretion signal peptide comprises an amino acid sequence having a sequence identity of at least 70% with the amino acid sequence encoded by polynucleotides 1 – 81 of SEQ ID NO: 2, or SEQ ID NO: 44.
- 20 10. An isolated polynucleotide encoding a polypeptide as defined in any of claims 1-9.
11. A recombinant expression vector or polynucleotide construct comprising a polynucleotide as defined in claim 10.
- 25 12. A recombinant host cell comprising a polynucleotide as defined in claim 10, or an expression vector or polynucleotide construct as defined in claim 11.
13. The recombinant host cell according to claim 12 which is a *Bacillus* cell.
- 30 14. A transgenic plant, or plant part, comprising a polynucleotide as defined in claim 10, or an expression vector or polynucleotide construct as defined in claim 11.

15. A transgenic, non-human animal, or products, or elements thereof, comprising a polynucleotide as defined in claim 10, or an expression vector or polynucleotide construct as defined in claim 11.

5 16. A method for producing a polypeptide as defined in any of claims 1 - 9, the method comprising: (a) cultivating a recombinant host cell as defined in claim 12 or 13, or a transgenic plant or animal as defined in claims 14 or 15, to produce a supernatant comprising the polypeptide, and optionally (b) recovering the polypeptide.

10 17. An animal feed additive comprising at least one polypeptide as defined in any of claims 1 - 9; and
(a) at least one fat-soluble vitamin, and/or
(b) at least one water-soluble vitamin, and/or
(c) at least one trace mineral.

15 18. An animal feed composition having a crude protein content of 50 to 800 g/kg and comprising at least one polypeptide as defined in any of claims 1 - 9, or at least one feed additive of claim 17.

20 19. A composition comprising at least one polypeptide as defined in any of claims 1 - 9, together with at least one other enzyme selected from amongst phytase (EC 3.1.3.8 or 3.1.3.26); xylanase (EC 3.2.1.8); galactanase (EC 3.2.1.89); alpha-galactosidase (EC 3.2.1.22); protease (EC 3.4.--), phospholipase A1 (EC 3.1.1.32); phospholipase A2 (EC 3.1.1.4); lysophospholipase (EC 3.1.1.5); phospholipase C (3.1.4.3); phospholipase D (EC 3.1.4.4); and/or beta-glucanase (EC 3.2.1.4 or EC 3.2.1.6).

25 20. A method for using at least one polypeptide as defined in any of claims 1 – 9, for improving the nutritional value of an animal feed, for increasing digestible and/or soluble protein in animal diets, for increasing the degree of hydrolysis of proteins in animal diets, and/or for the treatment of vegetable proteins, the method comprising including the polypeptide(s) in animal feed, and/or in a composition for use in animal feed.

30 21. A method for using at least one polypeptide as defined in any of claims 1 – 9, comprising including the polypeptide(s) in a detergent formulation.